Competent cells

Project: InterLab Measurements
Authors: Laura Laiho
Dates: 2016-07-05 to 2016-07-13

TUESDAY, 7/5

Prepared agar plates

WEDNESDAY, 7/6

Testing the competent cells according to the iGEM protocol:
iGEM protocol for competent cells was used: http://parts.igem.org/Help:Competent_Cell_Test_Kit.

TOP10 *Escherichia coli* competent cells and SOC medium from Gibson assembly were used.

THURSDAY, 7/7

Testing the competent cells according to the iGEM protocol:
iGEM protocol for competent cells was used: http://parts.igem.org/Help:Competent_Cell_Test_Kit.

TOP10 *Escherichia coli* competent cells and SOC medium from Gibson assembly were used.

*One difference in methods from Wednesday was that we added plasmids onto cells not other way around.

MONDAY, 7/11

Prepared agar plates for transformation
20 plates, 15 for iGEM plasmids

TUESDAY, 7/12

Testing the competent cells according to the iGEM protocol for the 3rd time.
iGEM protocol for competent cells was used: http://parts.igem.org/Help:Competent_Cell_Test_Kit.

TOP10 *Escherichia coli* competent cells and SOC medium from Gibson assembly were used.

Testing the competent cells also with another plasmid, pJR18, for control. Three different concentrations of DNA were used: for control 500 pg/ul, 50 pg/ul and 5 pg/ul. and 2 plates of each. Each concentration was plated on two ampicillin plates.

WEDNESDAY, 7/13

Counting colonies according to the protocol with excel sheet, transformation efficiency was quite low (average 1.8E7 cfu/uL and weighted 1.6E7 cfu/uL), but good enough for transformations.

Table 1.

compcell.png

$V_{DNA\ transformation}$ [µl]	1
V_{total} [µl]	251
V_{plated} [µl]	20

DNA concentration [pg/µl]	0,5	5	10	20	50
# of colonies	1	0	9	51	54
# of colonies	0	7	23	32	95
# of colonies	2	6	9	15	33
average # of colonies	1,0	4,3	13,7	32,7	60,7
efficiency	2,51E+07	1,09E+07	1,72E+07	2,05E+07	1,52E+07

Results	efficiency
average	1,8E+07
weighted	1,6E+07

Transformation

Project: InterLab Measurements
Authors: Saara Hiltunen
Dates: 2016-07-14 to 2016-08-05

THURSDAY, 7/14

The first day of the InterLab study. The DNA tubes were empty so they were resuspended in 200uL of dH2O and vortexed.

Transformation of TOP10 competent cells was performed according to the iGEM protocol:
http://parts.igem.org/Help:Protocols/Transformation.
Insted of pipetting 1uL of resuspended DNA, 5uL was pipetted. Following plasmids were transformed:

Device 1: J23101+I13504
Device 2: J23106+I13504
Device 3: J23117+I13504
Positive control
Negative control

TUESDAY, 8/2

Transformation had to be done again because of the LUDOX problem. Transformation of TOP10 competent cells was performed according to the iGEM protocol: http://parts.igem.org/Help:Protocols/Transformation.
Following plasmids were transformed:

Device 1: J23101+I13504
Device 2: J23106+I13504
Device 3: J23117+I13504
Positive control
Negative control

1 ul of plasmids were used for transformation instead of 5 (which was indicated in the exceptional protocol which stated that one should use 5 ul after resuspending them if the plasmids are dried up)

WEDNESDAY, 8/3

The second day of the Interlab study. Preparing the liquid culture:

5ml of LB
Chloramphenicol working concentration 25ug/ml.

Two colonies from each plate were picked and inoculated. Liquid cultures were grown overnight at 37°C 230 rpm.

Also restreaking the previous transformants if they happen to be too old.

THURSDAY, 8/4

Practicing the 3rd day protocol and preparing the liquid culture of the restreaked cells.

FRIDAY, 8/5

The 3rd day of the Interlab study. Measuring the OD600 of overnight cultures, diluting them to the OD of 0.02 and performing the assay:

Incubating cultures at 37°C 220 rpm and taking 100ul samples from each tube at 0,1,2,3,4,5 and 6 hours.
Measuring the ODs and FIs of each sample.

Table 1.

interlab7.png

Abs600
raw data

	positive control		negative control		device 1		device 2		device 3		media+chloramphenicol	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	col	
0 h	0,045	0,046	0,05	0,073	0,046	0,048	0,048	0,045	0,047	0,047	0,047	0,043
1 h	0,05	0,049	0,049	0,048	0,046	0,051	0,051	0,055	0,051	0,051	0,051	0,044
2 h	0,062	0,065	0,062	0,068	0,047	0,052	0,075	0,065	0,081	0,075	0,044	0,044
3 h	0,106	0,113	0,122	0,115	0,052	0,048	0,151	0,151	0,149	0,16	0,043	0,043
4 h	0,213	0,218	0,206	0,235	0,048	0,053	0,285	0,282	0,25	0,336	0,043	0,042
5 h	0,377	0,399	0,394	0,4	0,049	0,073	0,364	0,396	0,435	0,436	0,044	0,043
6 h	0,504	0,451	0,525	0,496	0,051	0,102	0,54	0,549	0,49	0,548	0,043	0,051
											blank average	0,044643
											correction factor	1,84375

blank substraction and correction

	positive control		negative control		device 1		device 2		device 3	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
0 h	0,000658	0,002502	0,009877	0,052283	0,002502	0,00619	0,00619	0,000658	0,004346	0,004346
1 h	0,009877	0,008033	0,008033	0,00619	0,002502	0,011721	0,011721	0,019096	0,011721	0,011721
2 h	0,032002	0,037533	0,032002	0,043065	0,004346	0,013565	0,055971	0,037533	0,067033	0,055971
3 h	0,113127	0,126033	0,142627	0,129721	0,013565	0,00619	0,196096	0,196096	0,192408	0,21269
4 h	0,310408	0,319627	0,297502	0,350971	0,00619	0,015408	0,443158	0,437627	0,378627	0,53719
5 h	0,612783	0,653346	0,644127	0,65519	0,008033	0,052283	0,588815	0,647815	0,719721	0,721565
6 h	0,84694	0,749221	0,885658	0,83219	0,011721	0,105752	0,913315	0,929908	0,821127	0,928065

Table 2.

interlab9.png

Fluorescence
raw data

	positive control		negative control		device 1		device 2		device 3		media+chloramphenicol	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	col	
0 h	689	703	706	743	865	836	522	640	709	665	780	808
1 h	842	773	844	870	1030	1070	704	696	687	732	641	816
2 h	1059	987	975	1182	1325	1102	695	476	697	706	889	796
3 h	1685	1394	1549	1537	1164	1335	733	716	709	710	782	853
4 h	2825	2410	2694	3252	1418	1558	673	739	579	865	803	570
5 h	4611	4037	5109	5455	1704	2241	628	743	886	864	888	839
6 h	5218	4209	7076	6865	1840	4124	911	939	892	1072	942	897
											blank average	807,4286

blank substraction

	positive control		negative control		device 1		device 2		device 3	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
0 h	-118,429	-104,429	-101,429	-64,4286	57,57143	28,57143	-285,429	-167,429	-98,4286	-142,429
1 h	34,57143	-34,4286	36,57143	62,57143	222,5714	262,5714	-103,429	-111,429	-120,429	-75,4286
2 h	251,5714	179,5714	167,5714	374,5714	517,5714	294,5714	-112,429	-331,429	-110,429	-101,429
3 h	877,5714	586,5714	741,5714	729,5714	356,5714	527,5714	-74,4286	-91,4286	-98,4286	-97,4286
4 h	2017,571	1602,571	1886,571	2444,571	610,5714	750,5714	-134,429	-68,4286	-228,429	57,57143
5 h	3803,571	3229,571	4301,571	4647,571	896,5714	1433,571	-179,429	-64,4286	78,57143	56,57143
6 h	4410,571	3401,571	6268,571	6057,571	1032,571	3316,571	103,5714	131,5714	84,57143	264,5714

Figure 1.

inter-1.png

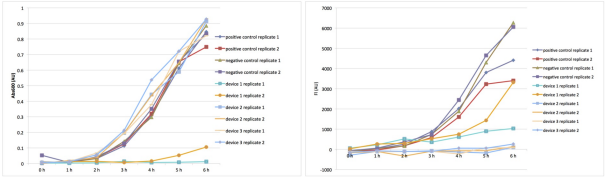


Table 3.

interlab11.png

FI/Abs600

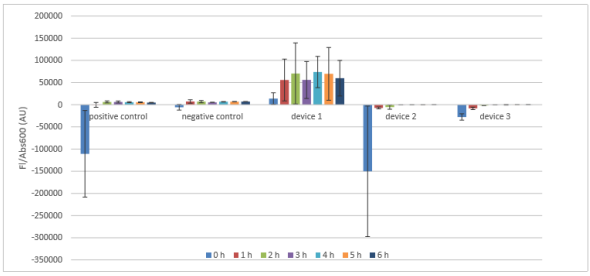
	positive control		negative control		device 1		device 2		device 3	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
0 h	-179851	-41734,2	-10268,9	-1232,29	23008,03	4615,939	-46113,2	-254264	-22648,2	-32772,5
1 h	3500,113	-4285,63	4552,376	10108,91	88949,15	22401,83	-8824,22	-5835,18	-10274,6	-6435,35
2 h	7861,059	4784,3	5236,242	8697,87	119091,9	21715,98	-2008,69	-8830,21	-1647,36	-1812,16
3 h	7757,384	4654,092	5199,368	5624,159	26286,65	85233,32	-379,552	-466,244	-511,56	-458,078
4 h	6499,73	5013,876	6341,369	6965,167	98642,63	48711,57	-303,342	-156,363	-603,307	107,1715
5 h	6207,04	4943,126	6678,139	7093,474	111604,3	27419,2	-304,728	-99,4552	109,1693	78,40104
6 h	5207,657	4540,144	7077,865	7279,075	88095,98	31361,72	113,4017	141,4886	102,9943	285,0786

average and SD

	average					SD				
	positive co	negative cc	device 1	device 2	device 3	positive co	negative cc	device 1	device 2	device 3
0 h	-110793	-5750,61	13811,98	-150189	-27710,3	97663,24	6389,865	13005,17	147185,1	7158,957
1 h	-392,761	7330,641	55675,49	-7329,7	-8354,98	5505,355	3929,061	47056,06	2113,57	2714,773
2 h	6322,679	6967,056	70403,96	-5419,45	-1729,76	2175,597	2447,74	68855,2	4823,541	116,5306
3 h	6205,738	5411,763	55759,99	-422,898	-484,819	2194,359	300,3728	41681,59	61,30067	37,81753
4 h	5756,803	6653,268	73677,1	-229,852	-248,068	1050,657	441,0917	35306,58	103,9301	502,3844
5 h	5575,083	6885,807	69511,77	-202,092	93,78517	893,7221	293,686	59527,88	145,15	21,75644
6 h	4873,901	7178,47	59728,85	127,4451	194,0365	472,0025	142,2767	40117,18	19,86043	128,7531

Figure 2.

interlab12.png



Calibration protocol

Project: InterLab Measurements
Authors: Saara Hiltunen
Dates: 2016-07-18 to 2016-08-01

MONDAY, 7/18

Preparing of the FITC solution according to the iGEM plate reader protocol: http://2016.igem.org/wiki/images/c/c5/InterLab_iGEM2016_Plate_Reader_Protocol_Updated_July.pdf.

20x PBS was diluted into H2O to get 1x PBS - 1 part PBS and 19 parts H2O -> 3,8ml of H2O and 200ul of PBS (4mL).
We made 4ml of 1xPBS, not 1 mL because we needed more of it later for the plate reader, but obviously for the FITC solution we used just 1 mL of 1xPBS.

So the protocol says "Prepare 2x FITC stock solution (500 µM) by resuspending FITC in 1 mL of 1xPBS"

n = m/M = C*V = 500uM * 1 mL = 0.5 umol (500uM FICT - 500 *10E-6 mol/L)
m= n*M = 0.5 umol* 389,382 g/mol=194.7 ug

In order to have 500uM concentration of FITC we take 194.7ug of FITC because it's a dry reagent.
We add 194.7ug of FITC into 1ml of 1xPBS and we get 2xFICT solution.

Then 2xFITC was incubated in 42°C for 4 hours.

Later to prepare 1xFITC solution we added 1 ml of 2xFITC into 1ml of 1xPBS.
Because 2xFITC got diluted by half its concentration became 250uM in 1xFITC solution.

Measured fluorescence of all samples in standard measurement modes in plate reader. Resulst can be found in table 1.

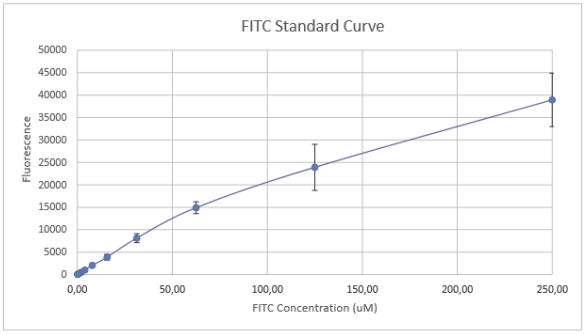
Table 1.

interlab2.png

	250,00	125	62,5	31,25	15,625	7,8125	3,90625	1,953125	0,976563	0,488281	0,244141	0
replicate 1	33023	24444	14334	8623	3108	1901	858	414	253	99	64	28
replicate 2	47229	16504	13329	6637	3506	1737	840	422	220	124	73	13
replicate 3	37590	27747	16189	8336	4372	2275	1128	546	281	161	100	20
replicate 4	37928	26927	15740	8792	4435	2250	1165	595	293	183	103	33
Mean	38942,5	23905,5	14898	8097	3855,25	2040,75	997,75	494,25	261,75	142,25	85	23,5
SD	5960,006	5130,262	1310,916	991,3664	654,0894	264,8602	172,5811	90,34886	32,48974	38,22194	19,44222	8,812869

Figure 1.

interlab3.png



MONDAY, 8/1

Obtaining a ratiometric conversion factor to transform absorbance data into a standard OD600 measurement by using LUDOX-S30 as a single point reference. Results can be seen in table 2. The same iGEM protocol as above was used.

Table 2.

interlab1.png

	LUDOX	H2O
replicate 1	0,045	0,037
replicate 2	0,045	0,037
replicate 3	0,045	0,037
replicate 4	0,045	0,037
average	0,045	0,037
corrected Abs600	0,008	
reference OD600	0,01475	
correction factor	1,84375	